

# LIFE-HISTORY AND PHOTOPERIODIC RESPONSES OF NYMPHS OF TETRAGONEURIA CYNOSURA (SAY)<sup>1</sup>

PAUL E. LUTZ<sup>2,3</sup> AND CHARLES E. JENNER

*Department of Zoology, University of North Carolina, Chapel Hill, North Carolina*

Many biological aspects of the Odonata are poorly known; for species in this country, problems involving regulation of development by seasonal changes have been neglected especially. Interest in seasonal regulation in the Odonata has been stimulated recently as a result of detailed investigations by Corbet on the dragonfly, *Anax imperator* (1956a, 1957a), and on other species (1955a; 1956b, 1956c; 1957b, 1957c). Summaries of his interesting ideas concerning seasonal regulation are to be found in two recent volumes (Corbet *et al.*, 1960; Corbet, 1963).

Life-history studies on dragonflies are neglected in spite of the many published reports on the subject. Most so-called life-history or life-cycle studies have been concerned with rearing nymphs to adulthood under artificial, and usually variable, conditions. A more desirable method of studying patterns of nymphal development is to sample regularly populations in nature throughout the year. This method has been utilized effectively by Corbet (1956b; 1957a, 1957b, 1957c) on several British species, by Eller (1963) on *Pachydiplax* in North Carolina, and in a limited way by Kormondy (1959) on *Tetragoneuria* in Michigan.

The experimental studies of Corbet (1956a), Jenner (1959), Lutz and Jenner (1960), Schaller (1960), and Montgomery and Macklin (1962) have shown that day-length is an important factor in regulating life-cycles of several species of Odonata. In preliminary experiments on *Tetragoneuria cynosura*, Jenner (1959) and Lutz and Jenner (1960) subjected over-wintering, last-stage nymphs to short and long photoperiods of 11 and 14 hours, respectively. On the longer photoperiod nymphal development was completed in one-third the time required by nymphs on the shorter photoperiod.

The present investigation was carried out to determine the pattern of development in *T. cynosura* nymphs in nature, and to study seasonal differences in photoperiodic responses. Since nothing is known about the threshold light intensity in Odonata photoperiodism or the minimal number of consecutive inductive photoperiods required for a response, attention was also given to these matters in this study.

## MATERIALS AND METHODS

Field studies were carried out at University Lake, an impoundment constructed in 1931 and located three miles west of Chapel Hill, North Carolina. The area

<sup>1</sup> Supported in part by grants from the University of North Carolina Research Council and the U. S. Public Health Service (E-356).

<sup>2</sup> Part of a dissertation submitted to the faculty of the Department of Zoology in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

<sup>3</sup> Present address: Department of Biology, University of North Carolina at Greensboro.

was selected because of accessibility and the high density of nymphs of *T. cymsura*. The shore line of the study area measured approximately 43 meters and contained numerous sedges, grasses, rushes and small shrubs. The bottom consisted primarily of abundant decaying vegetation resulting from dense rooted plants (chiefly *Sagittaria* sp. and *Saururus cernuus*) and bordering trees (*Platanus occidentalis*, *Alnus serrulata* and *Salix* sp.).

Twenty-one collections of nymphs were made at two- to four-week intervals from August 12, 1960, until August 16, 1961. Numbers of individuals varied from 10 to 116 per collection. Animals comprising nine collections were measured in the field and returned to their approximate habitat. Animals in the remaining collections were brought to the laboratory, allowed to come slowly to a temperature

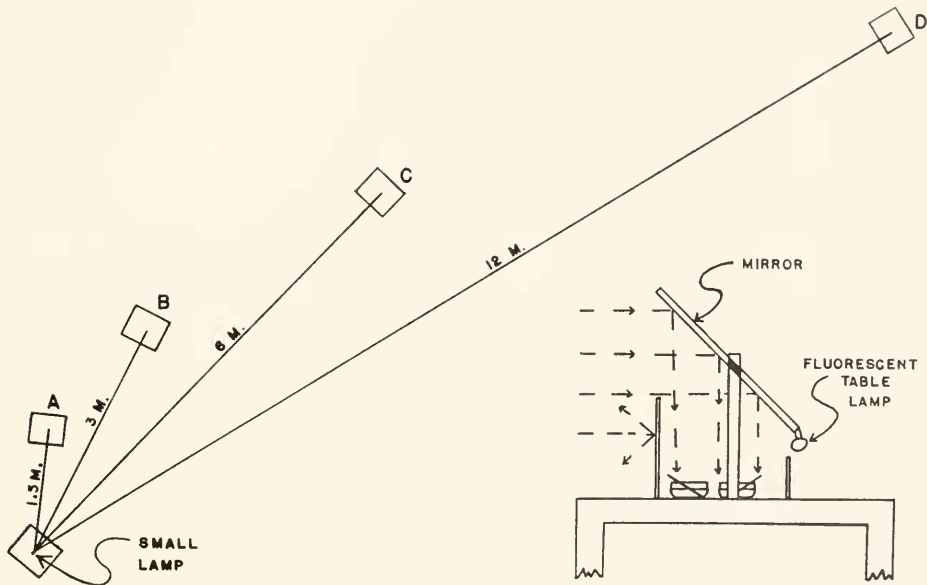


FIGURE 1. Plan of stations employed in light-intensity experiments and a diagrammatic representation of arrangements at each station.

of 22° C. and then measured, using a Bogusch measuring slide and binocular microscope. Measurements were made on total body length from the most anterior part of the labium (at rest) to the posterior ends of the lateral spines of the ninth abdominal segment.

All experimental animals were maintained in individual fingerbowls 10 cm. in diameter and were fed *ad libitum* on *Daphnia magna* with supplemental feedings of *Daphnia pulex* and tubificid worms. A 12-cm. length of dowel, 1 cm. in diameter, was added to provide a means of exit for the nymphs from the water at emergence.

All nymphs, except those utilized in light-intensity experiments, were housed in light-proof cabinets, each measuring 0.61 × 1.12 × 0.85 meters, in a controlled-temperature room at about 22° C. Photoperiods of 14 hours (long day) and 11 hours (short day) were employed. Light was supplied by two 30-watt daylight

fluorescent lamps operated by automatic time switches; intensities ranged from 270 to 430 lux. When the cabinets were closed, air from the room was circulated through them by a centrifugal blower *via* a system of light-proof ducts; temperature variations within the cabinets remained within  $\pm 1^\circ$  C. of room temperature.

Light-intensity experiments, utilizing the same facilities reported by Paris and Jenner (1959), were conducted according to the plan illustrated in Figure 1. A fairly constant temperature of about  $22^\circ$  C. was maintained, although occasional extremes up to  $\pm 8^\circ$  C. were recorded. Individual station lamps (15-watt, day-light fluorescent), controlled by a single time switch set for an 11-hour photoperiod, delivered an intensity of 375 lux at bowl level. Three additional hours of low-intensity light, provided by a small lamp of 6 watts and controlled by a separate timer, increased the duration of the photoperiod to 14 hours for the animals at Stations A, B, C and D (Fig. 1). Therefore, animals at Stations A through D received an identical 11-hour photoperiod, followed by three additional hours during which time the light intensity was progressively lower from Stations A to D. Nymphs which served as long-day controls were located on the table with the small lamp where they were subjected to an intensity of about 5.5 lux for the 14-hour period. Short-day controls were placed in a light-proof box near a station lamp and were covered manually at the termination of the 11-hour photoperiod and opened in the dark.

A second intensity experiment was executed in the same manner except that filters (eight thicknesses of #1 Whatman Filter Paper, which reduced intensities by 93%) were used at the four stations. The filters, located about 8 cm. above the nymphs, were in place only during the three-hour period when the 6-watt lamp was on.

## RESULTS

### *Seasonal cycle*

To illustrate the nature of the seasonal cycle of this species, results of the sampling program are given as relative length-frequency histograms in Figure 2a and 2b. Data for different size groups are given as percentage of the entire collection so that comparisons can be made more easily between samples of different sizes. The final four instars were identified readily on the basis of length, but earlier instars could not be separated by this method. Correct recognition of later instars was substantiated by rearing these individuals to emergence in the laboratory.

Most of the population had a one-year life-cycle, as is obvious from Figure 2. The collection of August 12, 1960, consisted of three instars with the antepenultimate being most abundant. Progressive development during August, September, and early October was demonstrated clearly and by October 22, all nymphs sampled were in the final instar. Winter collections consisted almost exclusively of last-stage nymphs.

By March 24, 1961, all last-stage nymphs showed morphological changes associated with impending emergence; these changes included swelling of the wing sheaths and the separation of the mesothoracic wing sheaths. The emergence period extended from April 5 until about May 13. Following this period a collection was made on May 18, employing approximately the same degree of effort as that used in the March 24 collection, but yielding only 10 nymphs. These nymphs obviously

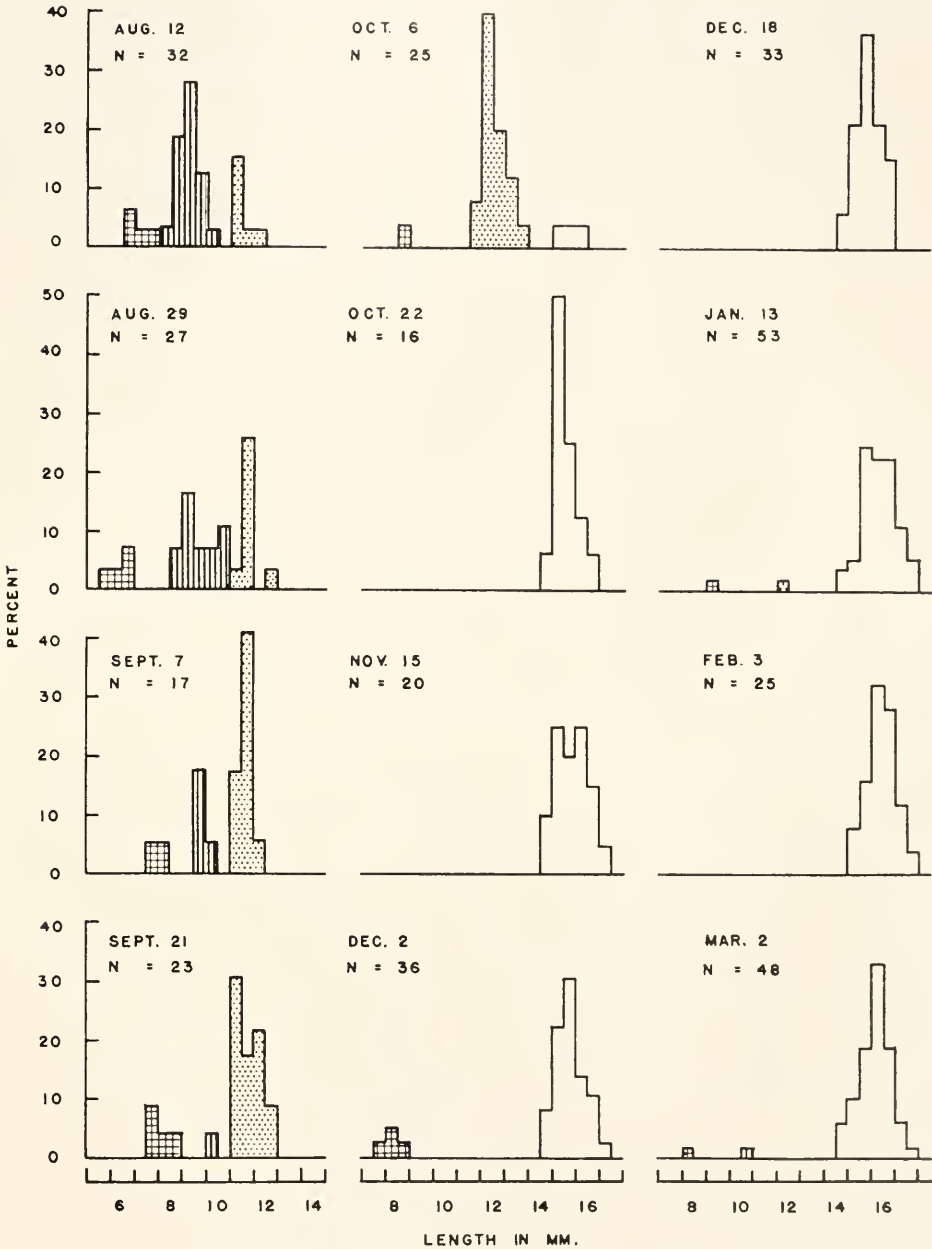


FIGURE 2A. Relative length-frequency histograms of *Tetragoneuria cynosura* nymphs collected in 1960-1961. Cross-hatched area = stage before antepenultimate instar; vertical lined area = antepenultimate instar; stippled area = penultimate instar; clear area = ultimate instar.

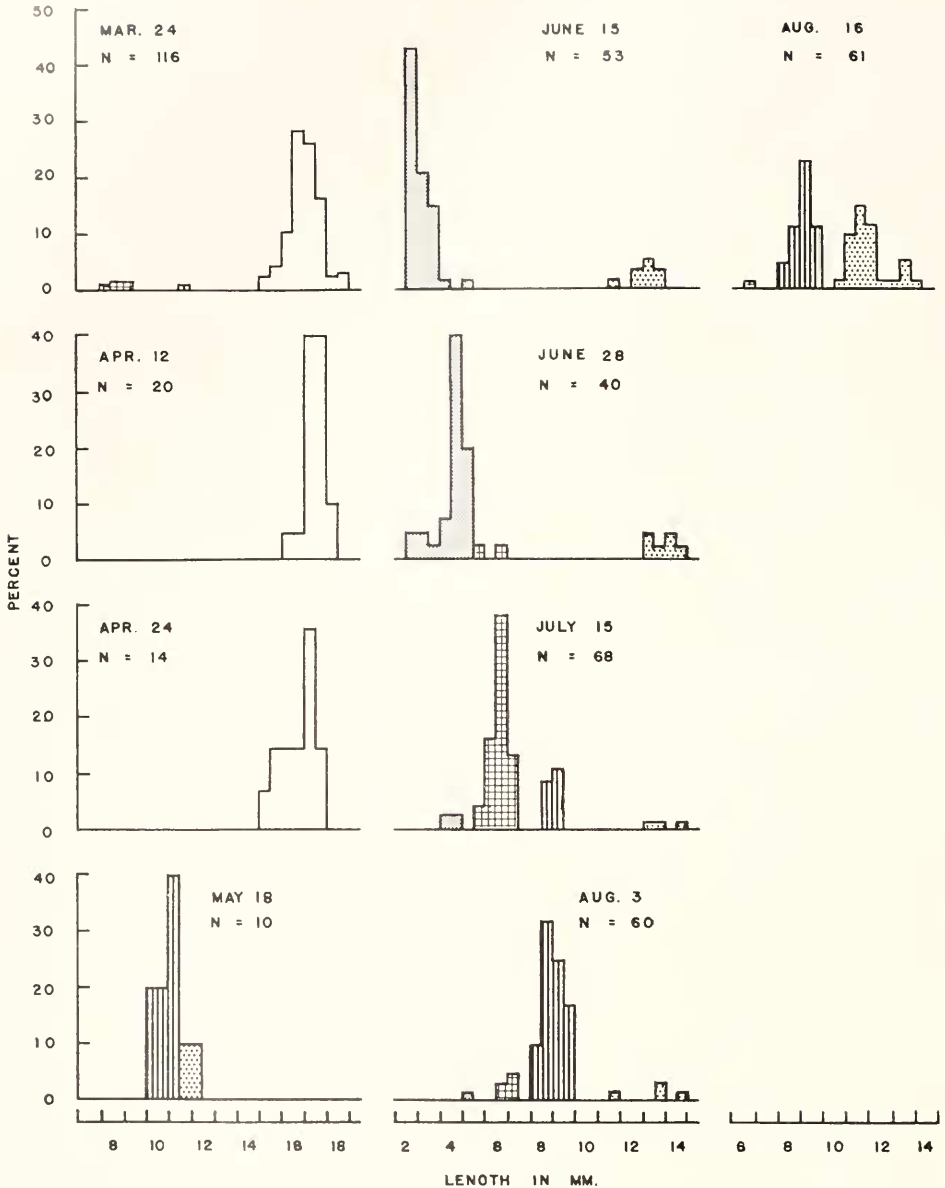


FIGURE 2B. Relative length-frequency histograms of *Tetragnocoria cynosura* nymphs collected in 1960-1961. Finely stippled area = early stages; cross-hatched area = stage before antepenultimate instar; vertical lined area = antepenultimate instar; coarsely stippled area = penultimate instar; clear area = ultimate instar.

had passed the winter in some stage short of the final instar. A small number of such individuals appeared in collections on December 2, January 13, March 2 and March 24.

The new generation was the dominant element in the June 15 collection, constituting 90% of the sample. This 0-year class developed rapidly during the summer, but remnants of the one-year class persisted as a small group in the penultimate stage. By August 16 recruitment into this latter stage had occurred from the 0-year class and a population distribution was again achieved, similar to the August collection of the previous year.

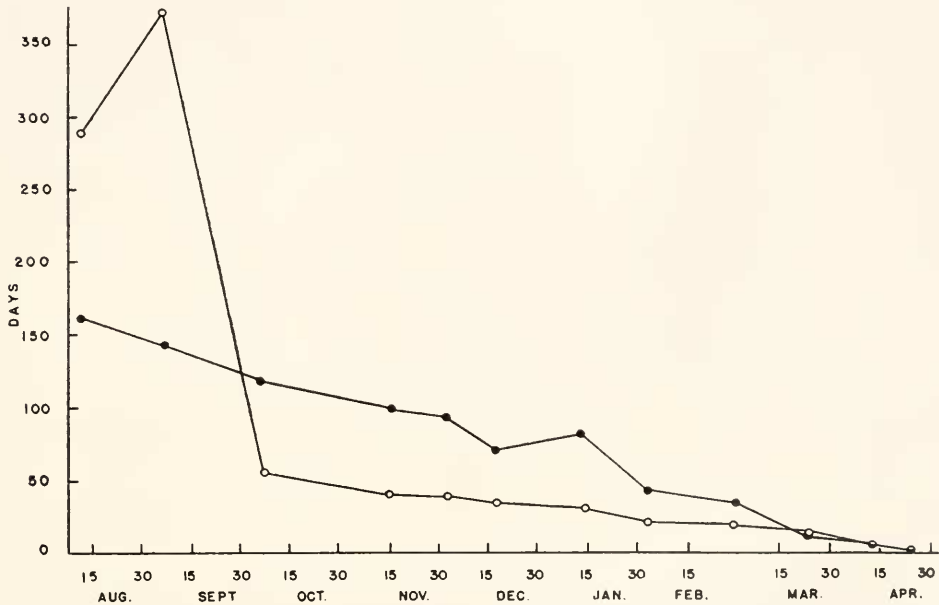


FIGURE 3. Average duration of time spent in the final instar in the laboratory by nymphs on 11- and 14-hour photoperiods. Open circles = 14-hour; closed circles = 11-hour.

Most individuals took only one year to complete their life-cycle, but a small part of the population required two years to undergo nymphal development and were, therefore, semivoltine. The percentage of retarded individuals in the samples varied from 0% to 15.2% and probably constituted 5-10% of the population.

*Seasonal changes in response to photoperiod*

In preliminary experiments Lutz and Jenner (1960) noted seasonal differences in response by nymphs to photoperiods of 11 and 14 hours. The response measured was time spent in the laboratory from collection to emergence. These observations suggested the desirability of initiating experiments throughout a more extensive period. Twelve experiments were started between August 12, 1960, and April 24, 1961, using the two contrasting photoperiods. All nymphs in the collections of August 12 and September 7, and all but two individuals in the October 6 collection,

spent the entire final instar in the laboratory. Data for nymphs from the remaining collections pertain to time spent in the laboratory from collection to emergence since all were already in the final instar when collected. Results of these experiments are shown in Figure 3.

An abrupt change in response was shown for nymphs placed on long-day conditions. Nymphs collected on August 12 and September 7 and kept on the 14-hour

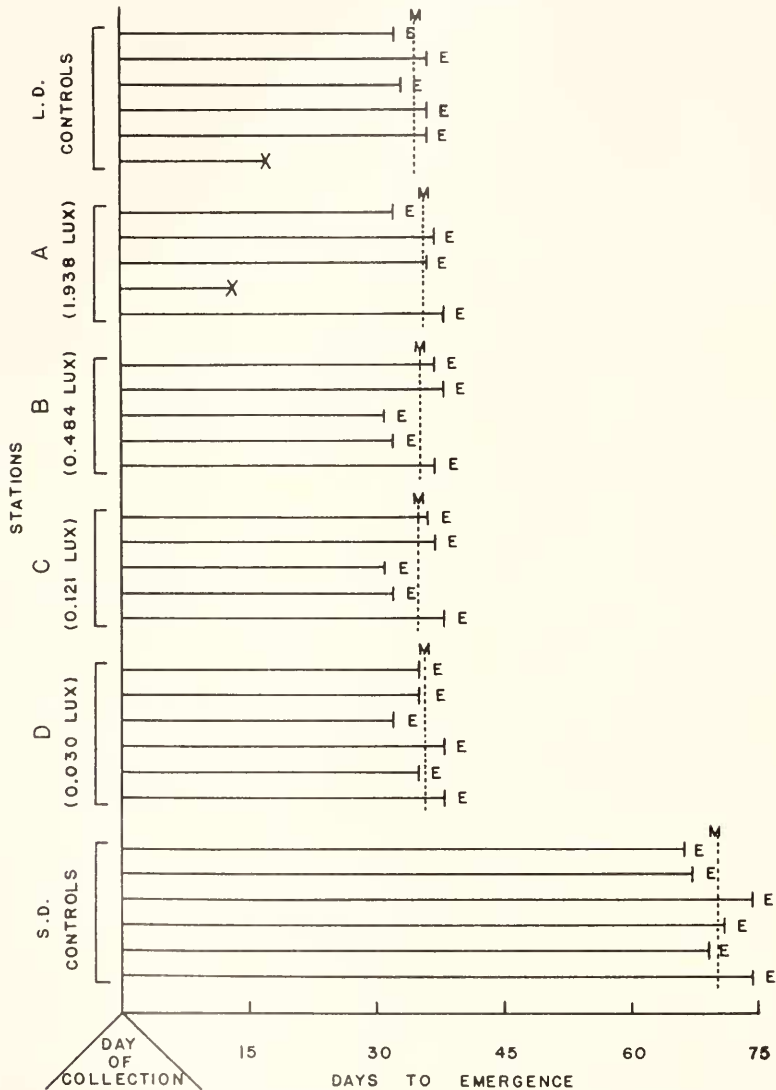


FIGURE 4. Days spent from collection to emergence by individual nymphs maintained on a basic 11-hour photoperiod plus three additional hours of low light intensity. M = mean; horizontal line ending with short vertical line followed by "E" = emerged here.

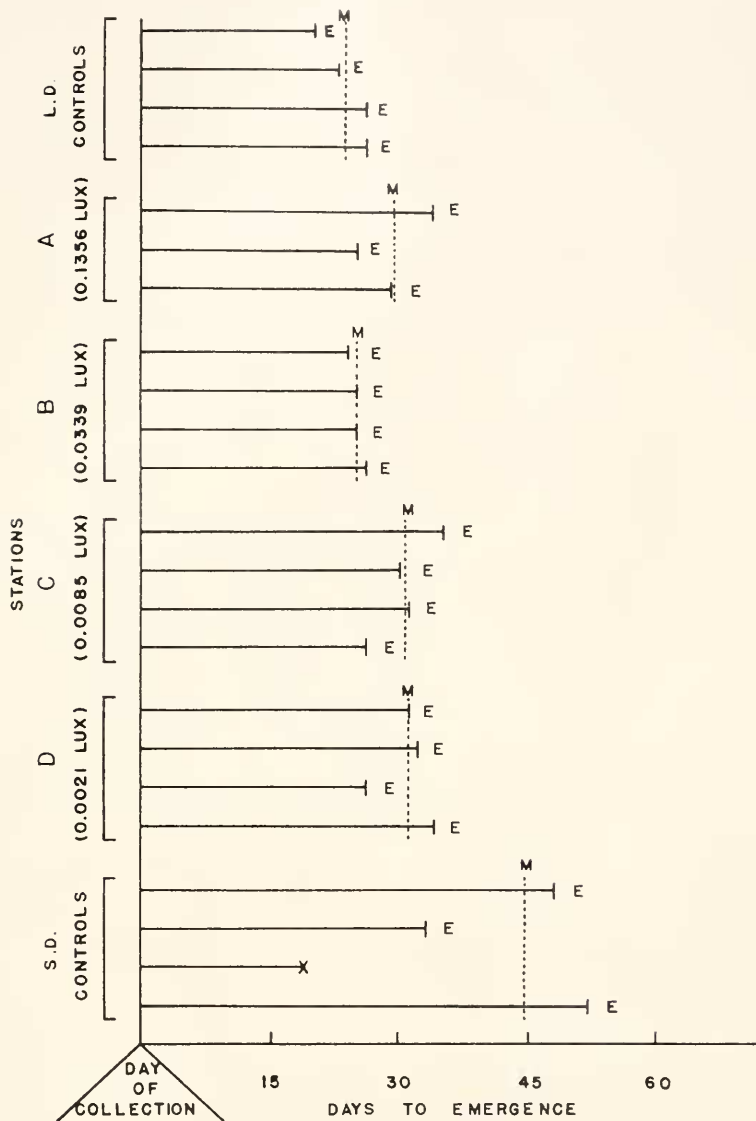


FIGURE 5. Days spent from collection to emergence by individual nymphs maintained on a basic 11-hour photoperiod plus three additional hours of very low light intensity. M = mean; horizontal line ending with short vertical line followed by "E" = emerged here.

day required approximately twice the time to pass through the final instar as did the short-day animals collected at the same time. In subsequent collections, however, the response of long-day individuals was much faster than that of nymphs on an 11-hour photoperiod. This dramatic reversal in response occurred during the period of the autumnal equinox.



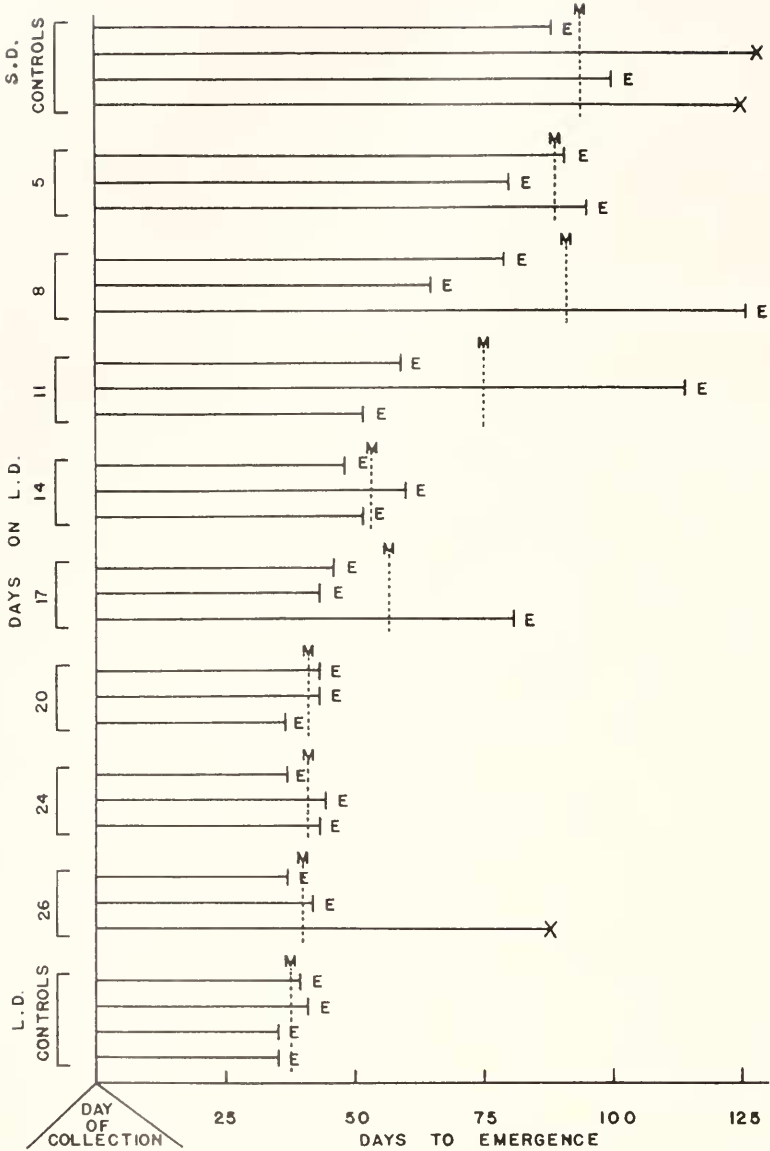


FIGURE 6. Individual responses of nymphs to an 11-hour day-length after being subjected to a varying number of long-day cycles. M = mean; horizontal line ending with short vertical line followed by "E" = emerged here.

The time required for short-day nymphs to emerge was progressively less throughout the entire experimental period. Following the autumnal equinox, differences in response to the two photoperiods decreased slowly until they were negligible for the last three collections.

*Light intensity experiments*

Two experiments were conducted in an attempt to determine the threshold light intensity for a long-day response. In the first experiment nymphs collected on December 18, 1960, were subjected to low experimental intensities ranging from approximately 1.94 to 0.03 lux. Days from collection to emergence for experimental and control animals are given in Figure 4. The results clearly show all experimental intensities to be above the threshold for a long-day response. A second experiment, therefore, was conducted, employing lower intensities that ranged from approximately 0.136 to 0.002 lux. Animals collected on February 3, 1961, were employed in the experiment; the results are given in Figure 5. Average days to emergence for nymphs subjected to these experimental intensities were closer to the mean for long-day controls than to that for the short-day animals. These results, although more variable than in the earlier experiment, indicate that the threshold intensity for a photoperiodic response in this species is exceedingly low, perhaps below 0.002 lux.

*Inductive cycle experiment*

Using final-stage nymphs collected on December 2, 1960, an experiment was conducted to determine the minimal number of 14-hour cycles (*i.e.*, 14 hours of light and 10 of darkness in each 24 hours) necessary for a long-day response. The animals were placed on a 14-hour photoperiod and, at intervals, three nymphs were transferred to an 11-hour photoperiod and maintained there until emergence. The results of this experiment are shown in Figure 6. The mean time to emerge for those nymphs subjected to five and eight long-day cycles was similar to the mean of the 11-hour controls. Animals experiencing 20 or more days on the 14-hour photoperiod responded similarly to the long-day controls. Nymphs subjected to 11, 14 and 17 long-day cycles exhibited a response intermediate between the two controls. Despite the small number of animals used and variations in response, the results suggest that more than 8, but less than 20, long-day cycles were necessary to produce a long-day response.

## DISCUSSION

Corbet (1963) has indicated that a diapause stage is a major factor in synchronizing nymphal development and emergence. Corbet *et al.* (1960) have classified British Odonata on the basis of position of the diapause stage, pattern, and duration of emergence periods, and mechanisms by which development and emergence are synchronized. They have designated as "spring species" those which emerge early in the season and have a diapause stage in the final instar. Emergence is characterized by an early peak due to the synchronizing effect of a final instar diapause. "Summer species" emerge later and a diapause stage (if there is one) does not occur in the ultimate instar. The emergence period in these forms is more widely dispersed temporally, due to a lesser degree of synchronization caused by the lack of a final instar diapause. Corbet (1957c) has postulated a theory of lower temperature thresholds which he believes would account for the degree of synchronization that is exhibited by "summer species."

Life-cycles of only a few dragonflies have been determined by regular sampling of natural populations. The present investigation is apparently the first published report for a species in this country. Corbet (1956b; 1957a, 1957b, 1957c) has studied life-cycles of several British species by this method, including a detailed study of *Anax imperator* (Corbet, 1957a). He observed a two-year life-cycle in *Anax*, although a small percentage developed precociously and was, therefore, univoltine. Employing a large sampling program, Eller (1963) has made an excellent study of *Pachydiplax longipennis* and found this "summer species" has a life-cycle of one year.

For *Tetragoncuria cynosura* the only information concerning the pattern of nymphal development in nature was given by Kormondy (1959), who reported a two-year life-cycle for this species in Michigan. His evidence was not satisfactory, however, since it was based on inadequate sampling and "extrapolation" from laboratory rearing. Our study conducted in North Carolina has shown conclusively that development for most nymphs of *T. cynosura* requires one year. The final instar was achieved generally by October and served as an over-wintering diapause stage. A well-synchronized emergence period occurred in the spring, and the position of diapause and emergence patterns characterize *T. cynosura* as a "spring species" in Corbet's classification. A small percentage of nymphs exhibited retarded growth and required two years for development. A pattern of development similar to that found in *T. cynosura* in this study has not been reported previously for a species of this insect order.

Corbet (1955b; 1956a) first reported a role of day-length in inducing diapause in Odonata. He concluded that the sign of change in successive day-lengths, *i.e.*, whether they are increasing or decreasing, influences the seasonal incidence of diapause in *Anax*. This conclusion should not be accepted without question since the results of his experiments, described as preliminary, were quite variable. Further, an assumption was made, as yet untested, that photoperiod was without influence once diapause was entered. Jenner (1959) and Lutz and Jenner (1960) demonstrated conclusively that an absolute photoperiod of 14 hours promoted development in diapausing, final-instar nymphs of *T. cynosura*, whereas an 11-hour day delayed development. Similar results have been reported by Montgomery and Macklin (1962) on *Neotetrum pulchellum*. The experiments by Schaller (1960) on *Aeschna cyanea* did not permit the separate effects of photoperiod and temperature to be determined.

Perhaps the most interesting aspect of this study concerns the observed differences in seasonal response to the two photoperiods. Experiments started in August and September showed that the duration of the final instar in nymphs maintained on long days was about twice that of short-day nymphs. A similar delay under long-day conditions must also occur in nature in the penultimate instar, which would account for the developmental arrest in the older nymphs (one-year class) occurring in summer collections. Day-length conditions during the period of the autumnal equinox must serve as a synchronizing agent and promote entry into the final instar by nymphs of both year classes. Following this period, short photoperiods must now induce delayed development in ultimate instar nymphs, thus insuring that they will enter the winter period in an appropriate stage of developmental arrest.

Delayed development was evident in long-day nymphs from the August 12 and September 7 collections and also by penultimate nymphs in nature during the summer. This developmental arrest may represent a phenomenon comparable to that known for certain birds which have a summer refractory period to photoperiodic induction. The role of day-length in terminating this refractory condition in *Tetragoneuria cynosura* has not, as yet, been clarified. An exploratory experiment, not reported in detail here, was conducted in which 5 nymphs of the collection of August 12, 1960, were subjected to alternating 15-day intervals of long and short photoperiods. These nymphs completed their development in less time than corresponding nymphs on either the long or short photoperiods. However, further experiments are required to clarify the critical factor or factors in operation.

The present study has demonstrated that nymphs respond to extremely low light intensities, probably below 0.002 lux. This value is lower than those reported by De Wilde (1962) in various insects and by Paris and Jenner (1959) in the midge, *Mctriocnemius*. The results of the inductive cycle experiment indicate that the effect of a long day is not irreversible initially, but requires at least eight long-day cycles to produce a long-day response.

#### SUMMARY

1. Most nymphs of *Tetragoneuria cynosura* required one year for the completion of nymphal development in nature. Rapid growth occurred in the summer months and by the end of October, the final instar was attained which served as an overwintering diapause stage; emergence occurred in early April and May. A small percentage of the population (5–10%) exhibited growth retardation and had a life-cycle of two years. These individuals spent the first winter in stages short of the last, the summer in the penultimate stage, and the second winter in the last stage.

2. Striking differences occurred in seasonal response to photoperiods of 11 and 14 hours. Durations of the final instar for nymphs collected in August and September were much greater on the longer day-length. Following the fall equinox period, a reversal in response occurred, with the longer photoperiod inducing more rapid development in fall and winter collections. Differences in rates of response became progressively less as time of emergence approached. These results were utilized in an attempt to explain the role of photoperiod in controlling seasonal nymphal development in this species.

3. The threshold light intensity necessary to elicit a photoperiodic response was found to be extremely low, probably below 0.002 lux. More than eight, but less than twenty, long-day cycles were necessary to induce irreversibly a response similar to that of the long-day controls.

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